

Keratoconus Progression in Patients With Allergy and Elevated Surface Matrix Metalloproteinase 9 Point-of-Care Test

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Purpose: To assess keratoconus (KC) progression in patients with allergies who also tested positive to surface matrix metalloproteinase 9 (MMP-9) point-of-care test.

Methods: Prospective comparative study including 100 stage I–II keratoconic patients, mean age 16.7 ± 4.6 years. All patients underwent an anamnestic questionnaire for concomitant allergic diseases and were screened with the MMP-9 point-of-care test. Patients were divided into two groups: patients KC with allergies (KC AL) and patients KC without allergies (KC NAL). Severity of allergy was established by papillary sub tarsal response grade and KC progression assessed by Scheimpflug corneal tomography, corrected distance visual acuity (CDVA) measurement in a 12-month follow-up.

Results: The KC AL group included 52 patients and the KC NAL group 48. In the KC AL group, 42/52 of patients (81%) were positive to MMP-9 point-of-care test versus two positive patients in the KC NAL group (4%). The KC AL group data showed a statistically significant decrease of average CDVA, from 0.155 ± 0.11 to 0.301 ± 0.2 logarithm of the minimum angle of resolution ($P < 0.005$) at 12 months; Kmax value increased significantly, from $50.2 \text{ D} \pm 2.7$ to $55.2 \text{ D} \pm 1.9$ on average. The KC NAL group revealed a slight KC progression without statistically significant changes. Pearson correlation test showed a high correlation between Kmax worsening and severity of PSR in the KC AL group.

Conclusions: The study demonstrated a statistically significant progression of KC in patients with concomitant allergies, positive to MMP-9 point-of-care test versus negative. A high correlation between severity of allergy and KC progression was documented.

Key Words: Allergy—Keratoconus—Keratoconus progression—MMP-9 point-of-care test.

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Keratoconus (KC) is a degenerative disease of the cornea, characterized by its progressive thinning leading to a cone-shaped cornea and irregular astigmatism resulting in a progressive loss of visual acuity.^{1,2} The etiology of KC is still poorly

understood, and many factors driving ectasia still remain unclear. However, atopy, eye rubbing, and inflammatory factors have been associated with KC.^{3,4} Previous reports suggest that progression of KC could be related to allergies.^{5,6} The first reported association between ocular allergy and KC was described by Hilgartner in 1937.⁷ Bawazeer et al.⁸ did a case control study finding a positive correlation between atopy, eye rubbing, and patient with KC tears exhibiting higher levels of proinflammatory cytokines, cell adhesion molecules, and matrix metalloproteinases (MMPs) compared with controls.^{9,10} MMP family enzymes mediate degradation of extracellular matrix proteins, typically in response to stress or injury.¹¹ Matrix metalloproteinase 9 (MMP-9) or gelatinase B is a 23 zinc and calcium ion-dependent enzyme, important for tissue remodeling in normal physiological processes like wound healing and bone development.¹² MMP-9 is also understood to play a pathogenic role in inflammatory disease. MMP-9 activity is regulated by epigenetic processes, cell–cell interactions, and cytokine-mediated pathways. Regulation of MMP-9 by IL-6 has been implicated in the degradation of type I and IV collagen fibers in corneal disorders.¹³ MMP-9 is then secreted in its proenzyme form bound to endogenous tissue inhibitors of metalloproteinases, and activated extracellularly by other proteases.¹⁴ This study evaluated the clinical and tomographic progression of KC in allergic patients with KC and positive surface matrix metalloproteinase 9 (MMP-9) point-of-care test while searching for a possible correlation between clinical severity of untreated, allergic conjunctivitis, grade of papillary sub tarsal response (PSR), and KC progression.

METHODS

The prospective study followed the tenets of the Declaration of Helsinki. The research included 100 patients with stage I to II KC according to Amsler-Krumeich classification,¹⁵ collected at the Ophthalmology Unit, Department of Medical, Surgical and Neurosciences of Siena University and at Siena International Crosslinking Center, Italy. Mean age was 16.7 ± 4.6 years (range 11–22). Informed consent was obtained from the subjects after explanation of the nature of the study. All patients underwent an anamnestic questionnaire for concomitant allergic diseases and according to questionnaire responses divided into 2 groups: group 1 including 52 keratoconic allergic patients (KC AL) and group 2 including 48 keratoconic patients negative for concomitant allergic diseases (KC NAL). Allergic diseases in the KC AL group consisted in 24 rhinitis (46.2%), 17 allergic conjunctivitis (32.7%), 6 asthma (11.5%), and 5 associations (rhinitis plus conjunctivitis) cases (9.6%).

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Grade of Papillary sub-tarsal response (PSR)	
	Absent (0)
	Low (1)
	Moderate (2)
	High (3)

FIG. 1. Clinical grading (from 0 to 3) of the papillary subtarsal response (PSR) assigned according to biomicroscopic evidence of subtarsal papillary hypertrophy recorded in the study cohorts. full color online

Patients underwent a full ophthalmologic examination, and the severity of allergy was clinically established in both cohorts by biomicroscopic examination and slit-lamp digital photography (SL990, C.S.O., Florence, Italy), evaluating the PSR and conjunctival hyperemia. The PSR was clinically graded from level 0 to 3 (0: absent, low: 1, moderate: 2, high: 3), Figure 1.

All patients were tested with the MMP-9 point-of-care test (InflammaDry test; RPS, Sarasota, FL), detecting the presence of MMP-9 (both the proform and active MMP-9) on the tear film based on a direct sampling microfiltration technology, Figure 2. The MMP-9, if present in the tear sample, is captured by the specific monoclonal and polyclonal antibodies for MMP-9 in a concentration ≥ 40 ng/mL. The InflammaDry test is composed of a pick-up-sample and one test stick, both individually wrapped in aluminum and sterile until the expiration date, to be kept between 25°C and 4°C. Tear samples were collected from the palpebral conjunctiva with the sample collector until the fleece

glistered, indicating saturation, Figure 2A. The test is performed by immersing the absorbent tip into the vial of buffer for a minimum of 20 sec, Figure 2B, ensuring the absorbent tip is not bent; immediately after the protective cap is repositioned and the device placed horizontally on a flat surface for at least 10 min, Figure 2C. The sampling fleece is then placed inside the test cassette with buffer solution. Within 10 min, if there is an MMP-9 antibody–antigen interaction on the immunoassay test strip, the result window will read positive with 2 lines (one blue and the other red) or negative with one blue line, as showed in the test response column of Figure 2.

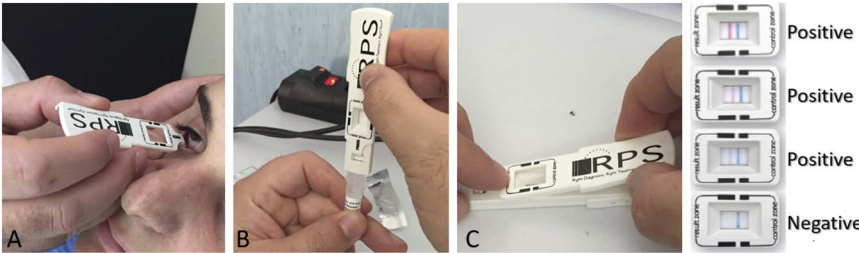
The test provides a qualitative (positive with MMP-9 level ≥ 40 ng/mL) response.¹⁶ The test must be read within 6 hr of its execution, after which results may skew. According to several studies,^{12,17–22} the average levels of MMP-9 (ng/mL) in human tears range from 3 to 40 ng/mL. A positive test result indicates the presence of MMP-9 ≥ 40 ng/mL. Keratoconus progression (Kmax, corneal thickness, and coma values) was assessed at baseline, at 3, 6, and 12 months with Scheimpflug corneal tomography (Sirius, C.S.O.; Costruzione Strumenti Oftalmici (CSO), Florence, Italy) and Spectacles Corrected Distance Visual Acuity (CDVA) determined. The ocular surface disease index (OSDI) questionnaire and Schirmer I tear test were performed in all cases excluding one patient for a mild dry-eye disease. Further, contact lens wearers were excluded from the study. Statistical analysis was conducted by the Mann–Whitney *U* test for nonparametric data (CDVA) and by the paired *t* test for parametric data (Kmax, coma value, and minimum corneal thickness). Box plot test analyzing Kmax values at 12 months compared with the severity of allergy (PSR grade), and Pearson correlation test was also performed between Kmax and corneal thickness at the 12-month follow-up in both groups attempting to establish a correlation between allergy, MMP-9 presence, and KC progression.

RESULTS

KC AL group patients showed 40 patients (77%) with high (grade 3) and 12 (23%) with moderate (grade 2) PSR. The KC NAL group revealed absent (grade 0) PSR in 38 cases (79%) and 10 (21%) low (grade 1) PSR cases.

Eye rubbing was reported by 64% of overall patients, all 52 cases of the KC AL group (100%) and 10 patients of the KC NAL group (20.8%). The InflammaDry test was positive in 42 patients (81%) of the KC AL group and in two patients of KC NAL group (4%). These two MMP-9 point-of-care test positive individuals belong to KC NAL eye rubbers and revealed a low (grade 2) PSR associated with a variable ocular itch. The other eight eye rub patients of KC NAL group were asymptomatic (without allergic symptoms).

FIG. 2. Clinical grading (from 0 to 3) of the papillary subtarsal response (PSR) assigned according to biomicroscopic evidence of subtarsal papillary hypertrophy recorded in the study cohorts. InflammaDry Test. (A) Tear sample collection. (B) Immersion of the test stick in the vial of buffer solution for 20 seconds. (C) Antigen-antibody reaction for MMP-9 detection requires 10 minutes. The right column shows the test answers: negative for blue line, positive with red line appearance. full color online



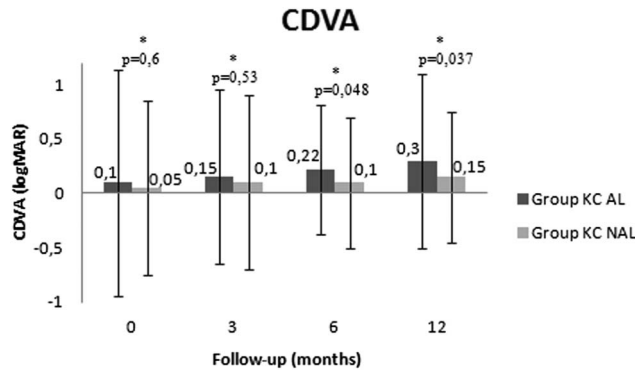


FIG. 3. Corrected distance visual acuity (CDVA). Only patients with KC AL showed a statistically significant progression of KC. The average CDVA decreased from a baseline value of 0.155 ± 0.11 to 0.301 ± 0.12 logarithm of the minimum angle of resolution (logMAR) (black line) at 12-month follow-up.

Patients with KC AL (indicated in the figure graphics with the black lines) at the 12 month of follow-up showed a statistically significant progression of KC. The average CDVA decreased from a baseline value of 0.155 ± 0.11 to 0.301 ± 0.2 logarithm of the minimum angle of resolution (logMAR) ($P=0.037$), Figure 3 (black line); average Kmax value, measured with tangential algorithm, increased from $50.2 D \pm 2.7$ to $55.2 D \pm 1.9$, Figure 4 (black line); minimum corneal thickness decreased from 454 ± 22 to $407 \pm 13 \mu m$, Figure 5 (black line). Coma value increased from 0.84 ± 0.03 to $1.14 \pm 0.02 \mu m$, Figure 6 (black line). Patients with KC NAL (indicated in the figure graphics with the gray line) after 1-year follow-up revealed a slight, even nonstatistically significant, tendency to KC progression. Average CDVA value decreased from 0.097 ± 1 to 0.187 ± 0.699 logMAR, Figure 3 (gray line); Kmax increased from $51.0 D \pm 2.8$ to $51.8 D \pm 2.2$ on average Figure 4 (gray line); minimum corneal thickness decreased from 464 ± 32 to $452 \pm 10 \mu m$ Figure 5 (gray line). Coma value passed from 0.94 ± 0.02 to $0.99 \pm 0.02 \mu m$ on average Figure 6 (gray line).

The correlation between Kmax and minimum corneal thickness average values at 12-month follow-up was assessed with the Pearson test. The scatter plot analysis showed a high correlation between Kmax increase and contemporaneous average minimum corneal thickness reduction in the KC AL group with a Pearson

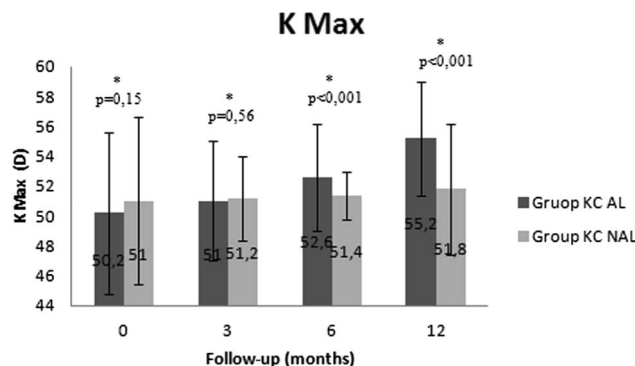


FIG. 4. Kmax. Average Kmax value, measured with tangential algorithm, increased significantly from $50.2 D \pm 2.7$ to $55.2 D \pm 1.9$ in the KC AL group (black line).

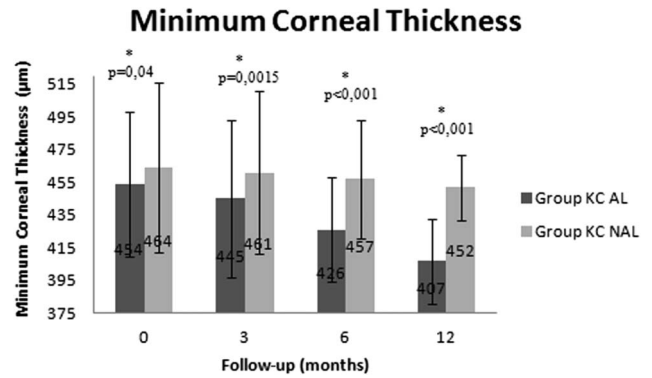


FIG. 5. Minimum corneal thickness. Minimum corneal thickness decreased significantly from 454 ± 22 to $407 \pm 13 \mu m$ only in the KC AL group (black line).

value of -0.85 , Figure 7A. On the contrary, the scatter plot analysis between the same instrumental parameters showed low data correlation in the KC NAL group with a Pearson value of -0.69 , Figure 7B.

Box plot analysis in overall patients showed that the highest Kmax increase at 12-month follow-up was correlated with the highest PSR. Each box includes minimum (min), first quartile (1Q), median (med), maximum (max), and third quartile (3Q). Major KC expressed by the highest Kmax values recorded at 12-month follow-up correlates well with the higher severity (grade 2–3) of PSR. Figure 8 box plot study shows that the increase of the average Kmax is greater in box 3 and 4 corresponding to the KC AL group patients showing the higher (grade 2 and 3) PSR. The 2-tailed Fischer exact test with 95% confidence intervals comparing type of KC (KC AL vs. KC NAL) to eye rubbing and InflammDry test confirmed a 2-tailed P value less than 0.001, Figure 9.

DISCUSSION

It is widely recognized that progressive KC represents the most frequent degenerative, noninflammatory, ectatic disorders of the cornea affecting pediatrics and young-adult patients up to age 40 years.² Beyond its degenerative nature, KC pathogenesis remains poorly understood and many factors driving ectasia progression unclear. We know that KC is characterized by a progressive stromal thinning correlated to increased keratocyte apoptosis driven by

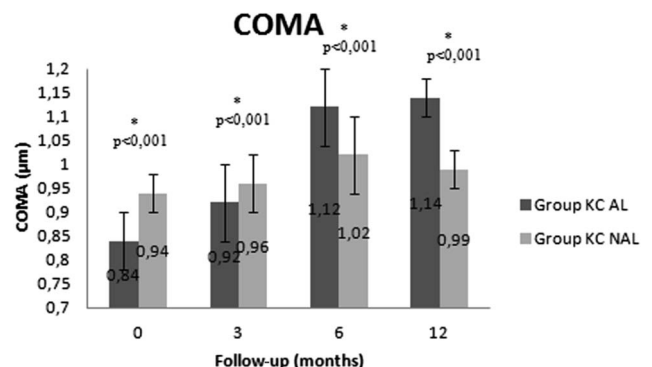


FIG. 6. Coma values. Coma value increased from 0.84 ± 0.03 to $1.14 \pm 0.02 \mu m$ in the KC AL group.

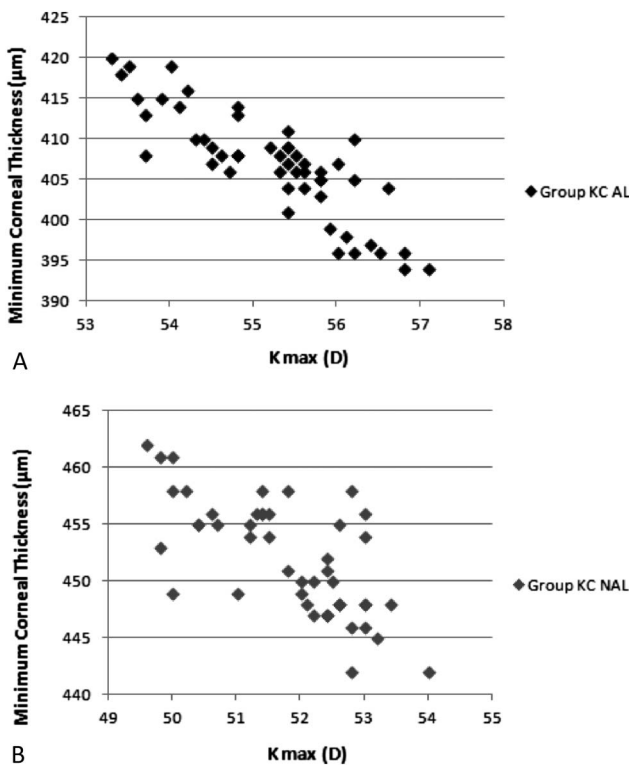


FIG. 7. There is a manifest correlation in KC progression that shows a contemporary increase of Kmax values to the decreasing of corneal thickness. The correlation between Kmax and minimum corneal thickness at 12-month follow-up is assessed by the Pearson test. (A) The Graph shows the highest correlation between Kmax increasing and corneal thickness reduction in KC AL group patients (Pearson value = -0.85). The graph (B) shows the lowest correlation between Kmax modifications and corneal thinnest point redo in the KC NAL group (Pearson value = -0.69).

genetic factors, but also as a result of increased expression of lysosomal and proteolytic enzymes and decreased concentration of protease inhibitors which result in altered collagen configuration.^{9,23}

Keratoconus evolution may be correlated in most cases with increased protease levels and activity, with the presence of inflammatory molecules in tears which have been found to be relevant in the pathogenesis. It has also been proven that eye rubbing increases the level of tear matrix MMP-9.⁴ Patients with keratoconus tears show a high rate of proinflammatory cytokines, adhesion molecules, and metalloproteinases (MMPs). An alteration of the corneal adjustment microenvironment that favors enzymatic activity imbalance is to be considered critical for the homeostasis of its connective tissue.¹¹ Enzymes like lysyl oxidase and the metalloproteinases are directly related to oxidative stress and to the degeneration of corneal collagen. There is a high genetic susceptibility associated with KC in whites and Asians determining alterations at the level of central corneal thickness, corneal curvature, collagen, and damage induced by oxidative stress. Keratoconus, as previously said, is characterized by a progressive thinning of the corneal stroma and corneal architecture decline, thus defining a primarily noninflammatory and degenerative disease. However, clinical evidence has linked several inflammatory molecules in KC modifying conventional paradigms so that several inflammatory pathways play a role in the pathophysiology of this disease: allergies, eye rubbing, and atopia are all associated with specific immunologic changes of corneal microenvironment. In KC, there is a high expression of IL-1α and IL-1β, and the corneal fibroblasts show high expression of receptors for IL-1α. Usually IL-1α is produced in the case of corneal trauma and inflammation, therefore, in the case of tissue damage or apoptosis. In KC, there is a loss of keratocytes, probably due to an increase in cellular apoptosis induced by the secretion of this interleukin. IL-1α and IL-1β both determine a cellular apoptosis at the level of the endothelium synergistically with the cytokine TNF-α. IL-1α also induces corneal oxidative stress only in KC, where SOD3 reduction has been specifically demonstrated. The thickness and the ectasia of KC corneas directly suggest a degeneration of corneal collagen by enzymes such as MMPs, whose activity is partly regulated by IL-1β. TNF-α, together with IL-6 and IL-1β, is considered one of the

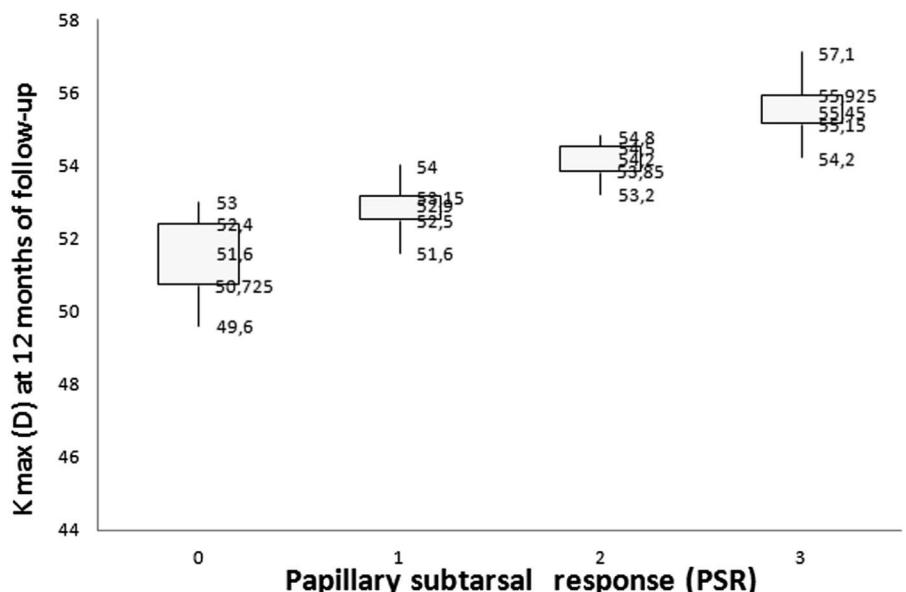


FIG. 8. Box plot: Kmax increase at 12 months of follow-up according to papillary sub tarsal response (PSR) in overall patients. Each box includes minimum (min), first quartile (1Q), median (med), maximum (max), and third quartile (3Q). Maximum increase of Kmax is correlated with highest PSR grade recorded in the KC AL group patients. Indeed the test shows that the increase of Kmax is greater in box 3 and 4 corresponding to KC AL group with grade 2 and 3 of PSR.

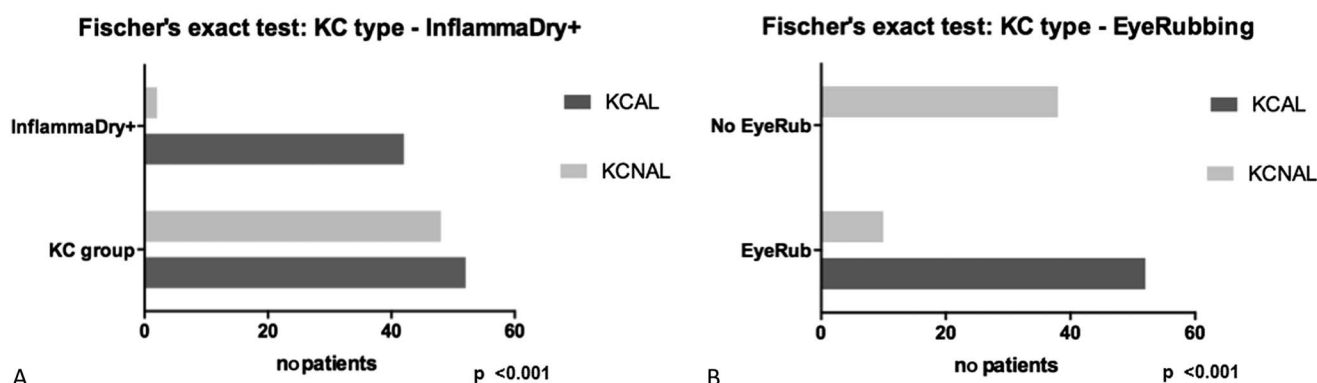


FIG. 9. The 2-tailed Fischer exact test with 95% confidence intervals comparing the type of KC (KC AL vs. KC NAL) to InflammDry test positivity (A) and eye rubbing (B) confirmed a 2-tailed P value less than 0.001 confirming the correlation between eye rubbing and MMP-9 point-of-care test positivity in patients with KC AL.

major factors involved in corneal inflammation, its levels are in fact increased in the tear film of patients with KC, and fibroblasts of these patients have a high expression of receptors for TNF- α . Unlike other interleukins analyzed, TNF- α can already be found in the subclinical stages of KC. This interleukin determines an increase in the levels of MMP-9, correlated with corneal thickness likely due to a degeneration of the stromal collagen.

TGF- β 2 controls the proliferation and differentiation of epithelial cells into endothelial cells, can interact with multiple types of collagen and stimulate the secretion of MMPs that influence the structure of the cornea and collagen distribution. TGF- β 1 is related to scar formation and tissue repair, evident in more severe KC cases.^{4,8–10} It has been hypothesized that this increase in protease activity may be further exacerbated during forceful eye rubbing as seen in allergic patients, contributing to the development and progression of the disease. The effect of allergies on KC progression was controversial. A few studies have reported an association ranging from 7 to 35%, whereas others showed none.⁷ However, we learnt from clinical evidence that allergy is highly present in the anamnesis of our keratoconic patients, and more recent studies have confirmed that there is a definite association between allergies and KC. The Dundee University Scottish KC study was a prospective observational study of 200 consecutive patients with KC.²⁴ They found atopic diseases including asthma (23%), eczema (14%), and hay fever (30%) present in the patients with KC.

In our study, the rapid progression of KC was statistically significant in the eye rubbing allergic patients (KC AL group) positive to elevated surface MMP-9 point-of-care test versus not-allergic (KC NAL) patients. Pearson correlation test showed a high correlation of Kmax increasing and contemporary optical thinnest point decreasing in patients with KC AL compared with KC NAL ones. Box plot analysis in overall patients confirmed that the increase of average Kmax was greater in box 3 and 4, corresponding to KC AL group patients showing grade 2 and 3 sub tarsal papillary response. The study demonstrated that a concomitant allergy may increase KC progression, especially in severe untreated eye rubbers cases. The MMP-9 point-of-care test was easy to perform and represented a noninvasive tool to indicate which patients have a higher risk of KC progression because of a higher level of MMP-9 on the ocular surface, with a specificity and sensitivity slightly under 80%. Eye rubbing may precipitate the

onset and exacerbate the progression of KC; hence, patients should be advised to avoid eye rubbing. According to our research, results, and clinical experience, careful monitoring and specific treatment for patients with KC suffering allergies is paramount because of higher risk of KC progression. A limitation of the study could be the exclusion of contact lenses wearers because contact lenses are the primary form of visual correction for patients with KC.²⁵ Contact lenses and any comorbidity that is, inflammatory in nature may add synergistically to other forms of KC-related inflammation and exacerbate its pathogenetic processes.²⁶

The association between pediatric age, allergies, and eye rubbing may represent a dangerous situation stimulating faster progression. These individuals are at higher risk of progression, possibly requiring corneal transplant, thus preventive measures and properly timed regimens of antihistaminic drugs, steroids, and particularly corneal collagen crosslinking therapy^{27–30} are key. Ophthalmologists and pediatricians should be alert and watchful of these particular conditions which require close monitoring and prompt treatment to prevent corneal deterioration and associated visual decay.

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